THE INCREASED RATIO OF STROMAL TO EPITHELIAL MALIGNANCIES IN MTF PATIENTS

by

Dinorah Jaime

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As members of the Master's Committee, we certify that we have read the thesis prepared by Dinorah Jaime, titled *The Increased Ratio of Stromal to Epithelial Malignancies in MtF Patients* and recommend that it be accepted as fulfilling the dissertation requirement for the Master's Degree.

_____ Date: April, 25, 2019
Diana Darnell

_____ Date: April, 25, 2019
Ghassan Mouneimne

_____ Date: April, 25, 2019
Joyce Schroedder

Final approval and acceptance of this thesis is contingent upon the candidate's submission of the final copies of the thesis to the Graduate College.

I hereby certify that I have read this thesis prepared under my direction and recommend that it be accepted as fulfilling the Master's requirement.

_____ Date: April, 25, 2019
Diana Darnell
Professor
Cellular and Molecular Medicine

_____ Date: April, 25, 2019
Ghassan Mouneimne
Assistant Professor
Cellular and Molecular Medicine

STATEMENT BY AUTHOR

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Abstract

Currently the literature on the risk of breast cancer, comparing MtF patients versus cisgender women, does not show a greater risk for MtF patients. MtF patients are not at a greater risk to develop breast cancer than ciswomen. While the most common type of breast cancer in ciswomen is seen in the epithelial component (12% incidence) of the mammary tissue, herein this specific population, there are case reports (3) of stromal lesions that reflect an increase in the stromal versus epithelial (20 cases reported) ratio of breast malignancies(3:20). The various variables that affect transwomen, like barriers to health or lack of proper education within in the medical community are addressed, in order to conceptualize the current data. The increased ratio of stromal to epithelial malignancies in MtF transgender individuals found in current literature could be attributed to the small sample size or truly a potential health hazard.

The Increased Ratio of Stromal to Epithelial Malignancies in MtF Patients

Introduction

The intake of estrogen by a Male-to-Female (MtF) patient is to ultimately drive breast development and cease prominent male traits, such as facial hair or muscle tone. The connection between long-term exposure to estrogen and the increased risk of neoplasia in the breast in MtF patients is scantily understood. Currently the literature on the risk of breast cancer, comparing MtF patients versus cisgender women, does not show a greater risk for MtF patients. MtF patients are not at a greater risk to develop breast cancer than ciswomen.

However, these results pertain to epithelial breast cancer, but not to stromal lesions, which make up less than 1% of breast cancers in the general population. In in the very small sample of breast cancers that have been reported in transwomen, stromal cancers make up 14%. Therefore, we need to understand the cellular and molecular mechanism of stromal vs. ductal and other epithelial malignancies in MtF patients, and decide if this is an anomaly based on small sample sizes or really a potential health concern.

Background

In order to conceptualize the distinct developmental path of an MtF patient's breasts resulting in their potential increased risk for neoplastia, we must first establish the physiological and anatomical development of breasts in general. There is no sex-related difference in breast development prior to puberty, thus embryonic, fetal, neonatal and childhood breast anatomy is similar for all typical individuals. Parenchymal and stromal elements comprise the human breast, with the parenchymal portion forming an epithelial

system of branching ducts that will eventually lead to the development of secretory acini, or milk-producing glands in the adult female breast with appropriate hormonal stimulation. The stroma or mesenchymal connective tissue contains mainly adipose tissue (fat), which will provide the environment in which the parenchyma can grow. The first stages of breast growth during embryogenesis are mostly hormone independent (Javed & Lteif, 2013), with the development of breast classified into two main processes: formation of a primary mammary bud and development of a rudimentary mammary gland.

The primary buds form into two separate areas of epithelial cells, with the surrounding mesenchymal cells differentiating into fibroblasts, vascular smooth muscle cells, capillary endothelial cells and adipocytes. The primary mammary bud vertically grows into the mesenchyme, which surrounds the primary bud. Secondary epithelial buds develop from the primary, with a narrow stalk and a bulbous end termed the terminal end buds. These epithelial cells grow vertically into a second stromal section, named the mammary fat pad (pre-adipocytes at this stage), in a process termed ductal morphogenesis. From the canalization and cleaving of the terminal end buds comes the rise of the lactiferous duct, which is the initial duct tree trunk. There are two layers of epithelial cells within the lactiferous ducts. The secretory function is within the layer of cells next to the lumen, and the basal layer becomes myoepithelial cells; together they form the preliminary epithelial ductal system. The basic framework of the gland is established by the end of the second trimester, with defined tubular structures surrounded by dense fibroconnective tissue. Breast tissue is apparent in both males and females at

birth, where the rudimentary gland is established and will grow parallel to normal body growth until puberty.

Compared to fetal breast development, which is considered to be sex- and hormone-independent (Javad & Lteif, 2013), pubertal and adult mammary gland development is sex-specific and hormone-dependent (Parent et al., 2003). During puberty in the female, there is primarily ductal elongation and side branching triggered through recurring estrous cycles (Parent et al., 2003). In puberty, estrogens are the key mitogenic stimulus for DNA synthesis and the increase of bud and duct formation. During puberty, the epithelium branches, forming additional bilayered ductal structures that consist of an outer basal myoepithelial layer of cells and an inner luminal layer. The luminal cell layer can be further divided into ductal luminal cells, lining the inside of the ducts, and alveolar luminal cells, that will secrete milk during (future) lactation. [The further development of the alveoli is dependent on another hormone, Progesterone.] At the culmination of the pubertal phase, while the underlying changes at the molecular level are occurring, extensive tissue remodeling impacts both stromal and parenchymal aspects of the breast. Thus, under the influence of estrogen, there is an increase in fibrous and fatty tissue of the stroma followed by ductal elongation and bifurcation of terminal end buds (Javed & Lteif, 2013).

Hormone Receptors

As with other steroids, estrogen acts via a nuclear steroid receptor, the estrogen receptor (ER), which upon activation by estrogen (ligand) forms a homodimer with another ER-ligand complex that leads to the activation of the transcription of specific genes that contain estrogen response elements. As there are two known estrogen

receptors, ER-α and ER-β, studies have been performed to determine which is responsible for mammary proliferation (Paterni, et al., 2014). Both estrogen receptors are found distributed along breast tissue, and both are known to play a role in breast development (Paterni, et al., 2014). Research on mice tracked the presence of both receptors in the female body throughout the key physiological stages that a female undergoes: pregnancy, lactation and post lactation, in order to hone in on the specific role of each receptor and establish the exact onset of expression for each (Paterni, et al., 2014).

According to Briskin and colleagues (2010), the two ERs relatively changed depending on the endocrine status of the mammary gland. During pregnancy, ER-α expression was less than 20-percent but during lactation it increased to 45-percent within the first week, then to 70-percent by the third week. Throughout various physiological stages depicted, ER-β was seen to be consistently present in more than half the epithelial cells. During the highly proliferative period of pregnancy, ER-β was the dominant receptor. During lactation, which is considered a non-proliferative period, both receptors were notable to be present but the ratio of ER-β to ER-α was reduced (Briskin, 2010). The message from this study is that between these receptors, there were no clear indications of which is key, α or β, as both are present during each physiological stage. In other words, both receptors were seen in the different physiological stages of breast development in female mice. Since there isn't specific information on the exact pathway of cis-females and MtF breast development in humans, it is understood to follow the same basic pathways as female breast development in puberty in other mammals. Thus, we will consider that both receptors may play a role in both cis-female and MtF human

breast development. However, as this data is purely correlational, it would be better to find a loss of function study to determine which of these receptors is actually necessary for breast development. For that we go back to mice.

Most of the information on estrogen receptors has been learned from studies in rodents. In a study done by Bocchinfuso et al. (2000), scientists targeted hormone receptor genes, and generated mice that were unresponsive to reproductive hormones, since their cognate receptors were lacking. All the mice were viable, but had reproductive deformities. ER-α is expressed in both the mammary epithelium and mammary stroma. The ER-α gene was disrupted through the insertion of a neomycin resistance gene (neo) into its first coding exon, which caused the elimination of ER- α.

In females that were ER-α -/-, (homozygous for the knock out), mammary gland development was equivalent to those of the wild-type (wt) cohort until the pubertal phase, from which point no additional development was seen through stereomicroscopy and histology. Thus, ER-α -/-, was shown to not be necessary for initial budding (hormone independent), but necessary for hormone dependent ductal elongation, side branching and alveologenesis of puberty. In contrast, mice that had ER-β knocked out (βERKO) demonstrated normal development of the mammary tissue (Couse et al., 1999). Thus, in puberty, estrogens are the key mitogenic stimulus and signal via ER- α, by stimulating DNA synthesis and increasing bud formation.

Similar to the ER- α receptor, the progesterone receptor (PR) is expressed in both the mammary stroma and mammary epithelium. Progesterone's main function is to induce proliferation of the endometrium every month during the menstrual cycle in preparation for pregnancy, but itis also the key stimulator of mammary epithelial DNA

synthesis and alveolar development. The elimination of ER- in the neo experiment (Bocchinfuso et al. 2000) also caused the nearly complete disappearance of the progesterone receptor (PR), because its expression is highly controlled by ER-. Typically, the presence of growth hormone (GH) induces the increase of estrogen, which through binding ER- sets the stage for progesterone action, through the induction of the PR (Kraus et al., 1990). This phase has been coined as "estrogen priming," which occurs in the majority of progesterone target tissues (Shyamala et al., 1990).

Progesterone levels were low in the ER-α knock out (αERKO) mouse, pituitary grafting and hormone replacement (exogenous estrogen doses) were used to determine estrogen's role regarding progesterone levels. In the presence of estrogen (grafting and hormone replacement), progesterone was shown to directly stimulate mammary gland development in the αERKO out mouse. Reduction of PR epithelial cells of females failed to side branch and form alveoli, which indicated that progesterone receptor signaling is necessary for side branching and alveologenesis. Furthermore, upon the exogenous introduction of estrogen and progesterone in αERKO mice, ductal and alveolar development was seen. Exogenous progesterone and estrogen in αERKO mice showed a 20-30% increase of PR mRNA, which could be due to indirect ER-β effects on progesterone. However, mammary gland development was successful in mice that were in PR deficient only in the stromal tissues, indicating that the receptor is necessary in epithelial cells but not in the stroma.

In conclusion, ER- α gene disruption leads to reduction in PR production due to the failure to induce the PR gen in mammary epithelia, which plays a role in the lack of ductal morphogenesis and alveolar development as seen in αERKO mice. Furthermore,

the reduced circulating levels of progesterone contribute to the deficient mammogenic signaling seen in the ER-α knock out mice. Additionally, because mice that had βERKO demonstrated normal development of the mammary tissue, this fails to support the earlier speculation about the importance of ER-β in breast development that led to the hypothesis that the ER-β role in breast development was necessary. Thus, the experiments discussed here together demonstrate that the ER- β and estrogen signaling pathway is not needed for breast development, but also reinforce that ER- α /E2 signaling is required directly for the morphogenesis of breast ducts, and indirectly through PR for the morphogenesis of breast side branching and alveologenesis.

Mammary Epithelial Tissue

Types and frequency of epithelial breast cancers (carcinomas). The mammary gland continues to undergo molecular changes throughout life that can lead to atypical growth. The most common breast cancers, carcinomas, arises from abnormal and proliferative behavior of cells (neoplasm) within the epithelia that will eventually extend into the periductal stroma or connective tissues (Modern surgical pathology CH 19). The exact etiology of breast cancer is one that continues to require research, but estrogen is considered to be a key growth hormone for both normal and neoplastic breast epithelium (Russo J. & Russo I., 2004). The exact mechanism by which estrogen promotes tumor proliferation is not fully understood, but the fact that 99% of epithelial breast cancer occurs in women (Allred et al., 2004) and that 92% of breast cancers express a type of estrogen receptor (ER) mRNA (Briskin, 2010), permit scientists to continue to look at estrogen's role in breast cancer development.

In their lifetime, one in eight (12%) cisgender women will be diagnosed with a form of epithelial breast cancer (National Breast Cancer foundation), making it the most common form of cancer overall, and the second leading cause of cancer death in women. Most breast cancers are epithelial in origin, with the most common derived from the terminal duct-lobular unit, the mammary ductal epithelium (Yerushalmi et al, 2009). Approximately 95% of carcinomas are in the ductal and lobular unit of the mammary (Ulster, 2009); 75% of them are invasive ductal carcinomas, and invasive lobular carcinoma is the second most common (5% - 15%) (Yerushalmi et al, 2009). Furthermore, statistics show that approximately 80% of cancers in the breast are ER-positive, 65% of those are PR-positive (American Cancer Society, 2019) and, ER- α is present in approximately 50-60% of breast cancers (Hinck & Silberstein, 2005). More specifically, 40% of ductal carcinomas (most common type of breast cancer) have ER- α expression. Thus, hormone receptors are major contributors to the field of breast oncology and define key steps for diagnosis and prognosis in patients.

Epithelial breast cancers are typically classified into three subcategories: estrogen receptor (ER)/ progesterone receptor (PR) positive, which is considered luminal; human epidermal growth factor receptor 2 (HER2) positive, and triple negative breast cancer (TNBC), ER/PR/HER2 negative (Hua, 2009). Luminal cancers can be further divided into two categories: Luminal A cancers are considered to have a low proliferation rate without the presence of HER2 receptors (Britten et al, 2013); Luminal B cancers, on the other hand, have a high proliferation rate and are HER2 positive; these are considered to be "HER2 driven cancers" (Sorlie et al, 2001). Most breast cancers are typically estrogen and progesterone receptor positive (Clarke et al, 2003). Both estrogen and progesterone

receptors are of importance when looking at pathological and normal development of the breast in ciswomen. In keeping with the focus of this review, which is breasts development and cancer in MtF patients, a closer look into estrogen receptors role in breast cancer proliferation and treatment is needed.

Epithelial Breast Cancer Treatment

The target in the search for treatment and prevention of developing breast cancer altogether is predominantly focused on estrogen and its receptor, ER, due to the fact that both are critical players in breast development (Joensuu et al., 2013) Along with ER, the two other receptors that are important when it comes to breast cancer are PR and HER2. Like ER, PR stimulates gene transcription of mammary epithelial DNA and is key in alveolar development (Russo J. & Russo I., 2004). Both, ER-α and ER-β cause upregulation of PR, which leads researchers to the deduction, that the combination of estrogen along with progesterone in menopausal treatment stimulates breast cancer (Russo J. & Russo I., 2004). Without hormone receptor expression, endocrine therapy (intake of drugs that inhibit and/or induce hormonal levels) is not an option for treatment, since the cells will not be able to take up the hormonal therapy and initiate a signaling (Russo J. & Russo I., 2004). Thus the absence of sex steroid receptors on cancer cells results in a higher risk for cancer progression and ultimately death (Allred et al., 2004).

The receptor that has not yet been covered, HER2, is found to be overexpressed in almost 25% of breast cancers (Warfard & Jasani, 2016). The presence of HER2, dictates the use of drugs that target HER2, such as Herceptin (Warfard & Jasani, 2016). HER2, PR, and ER detection are critical in defining the steps towards adequate treatment for breast cancer patients, since their presence allows not only the use of endocrine therapy,

but may diminish the need for chemotherapy. For example, triple negative ductal carcinoma lack expression of all three receptors and is also considered the most aggressive since the progression of such cannot be deterred through the additional use of endocrine therapy (Allred et al., 2004). However, HER2 and PR have been noted to play a role in the determination of mortality risk once diagnosed.

A study by Missmer et al. (2004) looked into the relationship between hormone levels and receptor status from examining 322 cases of breast cancer in postmenopausal women. Of the 322 cases found during their prospective analysis, 153 were ER and PR-positive (ER+/PR+) and 39 were ER and PR-negative (ER-/PR-). Each case was age matched to two control subjects that had their blood sample collected on the same month and time of day, to compare endogenous hormone levels available in blood plasma. Their results demonstrated a strong association between levels of circulating sex hormones and the risk of developing ER+/PR+ breast tumors. Thus, reinforcing the focus of scientist on ER and breast cancer links for future prevention and treatment. However, conflicting studies throughout time have emerged showing relationships between estrogen and breast cancer risks in postmenopausal women using hormone replacement therapy (HRT); that could also be contributors to the inability to uncover the mechanistic pathway of estrogen in regards to breast cancer risks.

Hormone Replacement Therapy

An infamous study done by *The Collaborative Group on Hormonal Factors in Breast Cancer* (1997*)* included 52,705 women with breast cancer and 108,411 without, from a pooled analysis of 51 epidemiological studies from 21 different countries. Their results concluded an increased risk of breast cancer development in current HRT users,

with a 2.3% (95% CI, 1.0% - 3.6%) risk increase in each year of usage. Women that used HRT for 5-years or more had a 35% increase risk of breast cancer, compared to nonusers. The increased risk disappeared in HRT users that had stopped using for at least, 5- years or more. It is important to note, The Hormonal Group stated that the average year of diagnosis among the 52,705 women with breast cancer was 1985, which at the time, HRT consisted of predominantly estrogen alone. The combinations of both estrogen and progestin in HRT began to increase in popularity around the 1990's, which is now the predominant type of HRT used today (Hall & Tressell, 2012). This study set an alarming wave throughout the medical community, but since then have been reevaluated and shown to be wrong.

Fournier et al. (2008) focused on estrogen and progestin in HRT during menopause, in 1,726-breast cancers found in their reanalysis of cohort data of 53,000 postmenopausal women. Their results demonstrated a 50% increased risk of breast cancer in HRT users that began using closer to menopause, versus nonusers. This result included the first 2-years of HRT usage, but in women that began HT at 3-years or more after menopause did not have an increased risk within usage of 2-years or less; however, did demonstrate a higher risk for longer usage, almost to that of women that began HT closer to menopause.

Additionally, studies have demonstrated that breast density increases after HRT is initiated in postmenopausal women (Greendale et al., 2003). The *Women's Health Initiative (WHI)* (2005), investigated the effects of progestin and estrogen versus estrogen only HRT, on breast density in a total of 16,608 postmenopausal women. There was approximately a 6% (95% CI, 4.6 – 7.5) increase of breast density in 75% of the women

using estrogen and progestin HRT for one year, compared to only the 0.9% (95% CI, 1.5 – 0.2) increase in the non-HRT users (placebo group). The overall consensus in the medical community continues to reflect the above studies, that combination HRT increases breast density by approximately 75% (even when used for only a short time); additionally, through increased breast density that is considered to have 4-6-fold increase risks of breast cancer (McTiernan et al., 2005).

In 2017, Manson et al., performed an in depth analysis of past HRT clinical trials. The study included an 18-year follow up, which prior studies lacked, and was published in the *Journal of the American Medical Association* (JAMA), a journal that requires high quality statistical modeling. The study demonstrated that HRT users did not have higher mortality rates, specifically of cancer, compared to non-users. More importantly, the study reflects, with analytical data, the current recommendations and beliefs of the medical community today; HRT should be an individual decision, based on the vast array of effects it has on human physiology.

Mammary Stromal Tissue

Hormone receptors on stromal tissue. In terms of epithelial malignancies, hormone receptors are key factors when clinicians are considering prognosis, treatment and survival (Conzen, 2008). Current research is heavily focused on finding preventive pathways and better treatment avenues for epithelial breast cancers in regards to hormone receptors, primarily ER- α and PR (Mueller et al., 2002). However, there is limited data on hormone receptors expression in the stroma, which has been established in rodents, to play a role in tumorigenesis and tumor growth (Sympson et al., 1995; Barcellos & Ravani, 2002). There are 48 different hormone receptors (Margolis et al., 2005) that are

involved in cellular proliferation and apoptosis (through regulation of target transcription gene expression). Knower et al. (2014) set out to identify which receptors were expressed in breast stromal tissue. They compared stromal tissues from normal and ER-positive cancerous patients to examine normal adipose fibroblast (NAFs) and cancer-associated fibroblasts (CAFs), respectively. Fibroblasts have been shown to be key mediators in tumor progression (Zamora et al., 2016). In their analysis, they found that the hormone receptors expressed in the cancerous stromal tissue are key players in regulation of aromatase. Aromatase is the main enzyme involved in the final steps of the biosynthesis of estrogen, in both males and females (Manna et al., 2016). The authors concluded that the disruption in aromatase regulation contributed to the increase of estrogen in ER-positive tumors.

Types and Frequency of Stromal Cancers (Sarcomas)

Breast malignancies that are non-epithelial, such as sarcomas, comprise less than 1% of all breast tumors (Ulster, 2009). Considering the small number of cases that appear as non-epithelial cancers in the general population, it is of no surprise that little is known of these types of tumors. Non-epithelial tumors of the breast include primary lymphomas, sarcomas, and hematological malignancies (Sullivan et al, 2004), with most cases having no known etiology, whereas others are possibly attributable to prior cancer therapy, such as radiotherapy (Chugh & Baker, 2004).

A retrospective study done in Northern Ireland, conducted over 14-years by O'Donnell et al. (2009) reviewed the prognostic outcome of non-epithelial breast cancers through the review of patient's medical records. Their investigation consisted of data from local hospitals, which demonstrated a total of 19 cases of non-epithelial cancers of

the breast from a total of 3,900 breast cancer cases found. Lymphomas (0.21%) were the main type and Sarcomas (0.13%) being the second type of non-epithelial cancers found from all breast cancers found. At the culmination of the 14-year study, nine patients were still alive, but nine others passed away due to their tumors, and another died from non-breast cancer related issues. The authors concluded that these types of cancers are rare compared to the 95% of epithelium breast cancers found, but that these carry a significant mortality rate. O'Donnell and coworkers encouraged researchers to continue the investigation of the outcome and etiology of patients diagnosed with non-epithelial malignancies.

Another retrospective study conducted over the course of four years (1994-1998) with a total of 363,801 cases of newly diagnosed breast cancer, found that approximately 0.4% of the tumors were primarily sarcomas (Young et al, 2004). A more recent investigation by Chirilia et al. (2017) looked into prognostic factors and outcome variability in breast sarcomas and malignant phyllodes (a type of non-epithelial tumor). The retrospective study was done over a 10-year period (2005-2015), and uncovered 67 non-epithelial cancers, with 40 of them being breast sarcomas (59.7%) and the rest (27) being malignant phyllodes (40.3%). The authors concluded there were no significant differences in outcomes because patients with sarcoma were treated the same as those with malignant phyllodes, and resulted in the same overall survival rate.

In this last study, patients with sarcoma were treated the same as patients with malignant phyllodes tumors, although, phyllodes are a specific subtype of non-epithelial malignancies. Primary sarcomas arise from mesenchymal tissues of the breast, with the annual incidence rate of 44.8 new cases per 10 million (Wang et al, 2014). Phyllodes on

the other hand, are derived from stromal connective tissue and have epithelial elements, while most phyllodes tumors are found to be benign (35-64%) there are cases of low-grade malignant tumors (borderline) and malignant (Chugh & Baker, 2004). Some scientists have attempted to separate phyllodes from sarcomas (Fields et al, 2008; Adem et al, 2004; Bousquet et al, 2007), due to the presence of epithelial elements in phyllodes, but this segregation is resisted by others because of the similar clinical and survival components of both (Chirilia et al., 2017;Terrier et al, 2000; McGregor et al, 1994). The difficulty in drawing the definitive line between sarcomas and phyllodes is further compounded due to the limited amount of research and number of cases available. For now, researchers and clinicians are trying to decide the appropriate course of action when it comes to phyllodes and sarcomas. The basic take home message, however, is that for ciswomen, these tumors are exceedingly rare, but have high mortality.

Stromal Malignancies in MtF Women

A recent worldwide literature review done by Joint et al. (2018) identified 20 cases of epithelial breast cancer in trans women. This demonstrates two notions. First, that only a few cases exist, which could be attributed to small population sizes or to lack of a representative sampling of data from this population. Secondly, of the current cases of breast cancers in MtF uncovered, the incidence overall is certainly not significantly greater when compared to rates in ciswomen (20:260,000; Dykes et al., 2018), and is hardly greater when compared to cismen (20:2,670; American Cancer Society, 2019). However, of the data we do have, current case reports on stromal lesions of the breast in MtF show a drastic difference in ratio of ductal to stromal, compared to that of ciswomen (Bryan et al., 2018; Richards et al., 2017; Tongson et al., 2017).

As mentioned, less than 1% of breast cancers are non-epithelial tumors in the general population. However, in the breast cancer literature we have three individual case reports of stromal lesions presented in MtF patients. Essentially, in the general population, there are 99 cases of epithelial breast cancer and only one of stromal. Where as in the MtF breast cancer literature, we find 20 cases of epithelial breast malignancies versus three stromal malignancies (Bryan et al., 2018; Richards et al., 2017; Tongson et al., 2017). This ratio seen in MtF of stromal to epithelial (3:20) malignancies is drastically different than the ratio seen in ciswomen (1:99). In regards to cismen, the ratio of ductal versus stromal is harder to define when looking at the available date. Similar to ciswomen, various studies (Muir et al., 2003; Buirhafour et al., 2007; Cutuli et al., 2010; Aagwar et al., 2014) report that invasive ductal carcinoma is the predominant cancer found in males, thus epithelial cancer. Furthermore, after a literature review of English data, Al-Benna et al. (2010) found that 98.5% of sarcomas are found in women, while only 1.5% occurred in men. By calculation, 1.5% of <1% or 0.015% off cancers overall should then be sarcomas in males.

MtF Case Reports on Stromal Malignancies

Overall, stromal malignancies are an extremely rare entity in the general population, but in MtF, these cases reported are a definite signal for further research in order to determine if they are anomalies due to limitations on current data and case reports, or if they are a cause for concern from the medical community when serving this population.

The first case is by Bryan et al. (2018) is of a 76-year-old MtF patient that developed an estrogen receptor positive mammary myofibroblastoma (MFB), a stromal

lesion, after receiving estrogen hormone therapy (HT) over 13 months. It was concluded that this was the first reported case of MFB in a transgender patient, after a literature review to identify all reported cases of breast neoplasia was conducted. This type of tumor is also called a benign spindle cell lesion with predominant myofibroblastic differentiation (Yu et al., 2018). Specifically, this case is not only unique because of the rarity of tumor type and origin but also due to the presence of ER positivity. ER positivity is seldom seen in this tumor type, because it is typically found in older postmenopausal ciswomen and older cismen, both of whom have low estrogen levels (Yu et al., 2018).

The second case is of a 38-year-old MtF transgender patient who presented with pseudoangiomatous stromal hyperplasia (PASH, a stromal lesion), along with lobular hyperplasia (an epithelial lesion). The patient had been taking estrogen for six months when she discovered a lump in her right breast. In this case, the patient had a history of breast cancer in her immediate family, where two of her sisters were diagnosed and one passed away from it. Case report did not include type of cancer patient's family members had. This case study highlights the importance of implementing medical pre-screening guidelines, since this specific individual had a higher risk for neoplastic development. Similar to the first case, PASH is a rare and benign mesenchymal lesion that is caused by proliferation of stromal myofibroblasts (Bowman et al., 2013). This lesion is extremely uncommon in cisgender females, with fewer than 200 cases reported since its discovery in 1986 (Jauno et al., 2010), and mostly presented in premenopausal women (Bowman et al., 2013). The etiology and pathogenesis of PASH is unclear, but they are considered to be due to hormonal factors, since it mostly occurs in hormonally active women, i.e.

premenopausal (Bowman et al., 2012). Another piece of evidence supporting the etiology of PASH being due to estrogen and progesterone levels is the fact that the largest masses of PASH were seen in women with the highest levels of hormonal activity (Brown et al., 2012).

The final case by Richards et al. (2017) of a 25-year-old MtF patient that presented with benign phyllodes tumor (PT) after taking estrogen intermittently since the age of 12. She began taking estrogen in the form of oral contraceptives from her cousin when she was 12, and at age 16 she was prescribed estrogen. Due to her insurance and financial situation she was not able to afford it from her provider, so she purchased from acquaintances at other times, thus her estrogen exposure overall is uncertain and incompletely documented. Benign phyllodes tumor (PT) is one of the two main types of fibroepithelial lesions, with fibroadenoma being the other. PT arises from proliferation of both stromal and epithelial factors but its neoplastic factor comes from the stroma. More recent studies have revealed that interaction between the stroma and epithelium is lost in PT, which can lead to an increase in mitotic activity in the stroma and overall stromal proliferation to progress into malignancy (Sapino et al., 2006). Malignant PT is more commonly seen in women of ages 45-49 and benign PT, as is the case with this patient, in younger generations. With regards to hormone receptors, neither malignant nor benign PT differ in its overall hormonal expression, however, ER expression was greater in benign PT, which are those typically found in hormonally active women.

Individually, each of these three cases denotes atypical pathological occurrences in their own realm, however, combined with the intake of feminizing hormones emphasize the need for further research. All three cases depict rare lesions from

unknown etiology but all three types of pathology have been attributed to possible connections to hormonal levels in ciswomen. When taken out of context of the specific population it was found in, the literature clearly implies aberrant hormonal levels are involved. There are other overlapping similarities between the three tumor types as well. PASH and MFB have been regarded as "closely related" due to their morphological and immunophenotypical similarities: both being produced from myofibroblasts, developing into solid spindle cell masses, and expressing ER and PR. In terms of clinical findings, imaging from mammograms and ultrasounds, practitioners often misdiagnose PASH as fibroadenoma, due to the presence of a dense mass during clinical examination, which more likely tend to be fibroadenomas (Bezic & Srlbjin, 2018); and as mentioned above, fibroadenoma and PT are both a type of fibroepithelial lesions. Histologically speaking, however, PT demonstrates eminent "leaf-like architecture and exaggerated intracanalicular stromal growth pattern" whereas fibroadenoma and PASH do not.

In addition, all three stromal tumor types have two additional intriguing similarities that reinforce the contention that each can be due to estrogen levels. All three lesions have been found at a very minute (less than 1%) percentage in men with gynecomastia, estrogen-induced pathological breast growth. More specifically, the abnormal hormone levels in the context of gynecomastia are an increase in estrogen or a decrease in androgen in the estrogen to androgen ratio.

The implication of hormones as the culprit is further strengthened by the second similarity, the presence of ER and PR in all three-tumor types. A study on PT used evidence from a PASH study by Bowman et al. (2012), which states "that 95% of the PASH tumors in our study stained positive for ER and/or PR receptors and a study from

Sapino et al. (2006), which declares "the presence of ER-β within the stromal cells and epithelial cells of fibroadenomas and phyllodes tumors," in order to stress their deduction of estrogen as the culprit for the mechanism of pathogenesis. Finally, a study on MFB "found a strong ER/PR nuclear labeling in 70-90% of the neoplastic cells in all cases of MFB" (Magro et al., 2000). The three tumor types individually paint a collective portrait where the protagonist is at center stage, estrogen and, in the context of the subpopulation that is the topic of this thesis, which further compounds our alarm at these findings.

Male Breast Cancer in XY Individuals

Gynecomastia. Gynecomastia is one of the most common breast conditions in males, 60-90% (Cuhaci et al., 2014) of male infants develop neonatal gynecomastia from exposure to high estrogen levels during pregnancy (Cuhaci et al., 2014), and at least 50-55% (M-2) of the male population will be affected in their post-natal lifetime. There is an increase in incidence of gynecomastia with age (Sansone et al, 2016), attributed to the decrease in testosterone that occurs in aging males (Golan, 2016) due to lower function in both testicular and hypothalamic-pituitary axes (Golan, 2015). The cause of gynecomastia remains unclear, but typically, the medical community attributes it to excess endogenous estrogen and/or a deficiency in androgens (Sansone et al, 2016). Although testosterone is the dominant sex hormone in males, estrogen is found at low levels in various points of development and it aids in spermatogenesis (Hess, 2003). Low levels of circulating estrogen come primarily from the conversion of androgens and testosterones through the enzyme aromatase, located in the testes (Hess, 2003) and adipose tissue (Lee, 2013).

Olsson et al. (2002) performed a prospective cohort study on 446 men from 1970-1979, to assess whether there was a correlation between gynecomastia and an increased

risk of cancer. Gynecomastia was confirmed through histological screenings. Their analysis demonstrated a significant increased risk for testicular cancer and squamous cell carcinoma of the skin in men with gynecomastia. Although their findings did not include breast cancer (presumably because none was found), it is important to examine further the importance of their findings between gynecomastia and testicular cancer.

According to recent studies, gynecomastia may be an early symptom of testicular cancer (Djaladat et al, 2019; Kolitsas et al, 2011; Dupar et al, 2003). Dupar et al (2003) were the first to report estrogen-caused gynecomastia due to a germ-cell testicular tumor. Germ-cell tumors (GCT) comprise 90% of testicular tumors (Olsson, 2016). Some secrete estrogen or human chorionic gonadotropin (hCG), which promotes the aromatization of estrogen from testosterone in the testes (Kratz et al, 2010). However, Dupar and similar studies may have mistaken correlation for causality between gynecomastia and aberrant estrogen levels. There are conflicting studies that demonstrate the opposite causal relationship of the Dupar study. In these studies, increased levels of estrogen cause the proliferation of testicular malignancy (Bouskine et al, 2008; Pais et al, 2003; Strohsnitter et al, 2001; Dieckman et al, 2001; Wein et al, 2000; Depue et al, 1983; Henderson et al, 1983). Since increased estrogen causes gynecomastia and increased estrogen is correlated with testicular malignancies, it is logical that gynecomastia and an increased incidence of testicular cancer will also correlate. The real take home message for our purposes is that XY individuals may have estrogen-sensitive neoplasms that correlate with increased estrogen levels.

Klinefelter syndrome. Two in 1000 newborn boys will have Klinefelter Syndrome (Bojensen et al., 2003); these individuals are born with at least one more X added to their

XY karyotype, typically seen as 47 XXY (Herlihy et al., 2011). Due to the genetic abnormality of the extra X, individuals affected present with testicular dysgenesis, sterility, gynecomastia and sometimes forms of psychological disorders (Herlihy et al., 2011). Various studies have been performed throughout history to examine the effects of this genetic anomaly. Wang et al. (1975) examined the endocrine profile of 19 patients with Klinefelter Syndrome (KS), found low levels of circulating free testosterone, but increased levels of luteinizing hormone (LH), follicular stimulating hormone (FSH) and estrogen. More importantly, scientist uncovered higher rates of testosterone converted into estrogen by peripheral organs. Another study by Hultborn et al. (1997) examined 93 cases of male breast cancer and established a 7.5% prevalence rate of Klinefelter Syndrome, which authors translated into a 50-fold increase risk for breast cancer in patients with Klinefelter Syndrome versus males with the typical XY karyotype.

Estrogen treatment for prostate cancer. Prostate cancer is sometimes considered to be an androgen-dependent malignancy, in which cases the treatment with estrogen is deemed to prevent further growth (Bosland, 2005). However, various studies have suggested the estrogens in prostate cancer treatment may lead to breast cancer in males. Schlappak et al. (1986) in a review of 19 cases of breast cancer in males found two patients (~10%) to have been taking estrogen for 12-years for their treatment of prostate cancer.

Obesity. Peripheral conversion of testosterone into estrogen by adipocytes has been hypothesized as the source of increased estrogen seen in obese males (Lee et al., 2013). Casagrande et al. (1998) conducted a case-control analysis of 75 cases of male breast cancers, to examine the risks associated with endocrine elements. From all the factors

measured, such as family history of cancer and lab analysis of serum levels, obesity was the only risk factor found. The authors stated that there was a doubling of risk for individuals that weighed 80 kg or more at 30-years of age, compared to those that weighed 60 kg or less of the same age. Casagrande and colleagues found lower levels of sex hormone binding globulin (SHBG), a protein that binds and sequesters estrogen, in obese men, which they believed suggested higher levels of free estrogen was circulating in obese men. Many studies following Casagrande et al.'s seemed to echo the same results: obesity is a risk factor for male breast cancer (Hsing et al., 1998; Ewertz et al., 2001; Johnson et al., 2002; Brinton et al., 2008), probably because high levels of estrogen are found in obese men.

MtF

A recent literature review done by Joint et al. (2018) uncovered a total of 20 breast cancers in MtF individuals, found in three cohort studies and 12 individual case reports. Cohort studies were done in a retrospective manner, with two cases found in a cohort study of 2,307 MtF individuals (Gooren et al, 2013), resulting in an incidence rate of 4.1 per 100,000 person-years, which was stated as being close to the expected rate in cismen (1 per 100,000py) but lower than the rate expected in ciswomen (130 per 100,000py; Gooren et al, 2013). Two more cases found in another cohort study that consisted of 5,135 transwomen (Brown et al, 2015), with an incidence rate of 0.03 per 100,000 person-years, and stated to not be significant in regards to expected rates in ciswomen (Brown et al, 2015). The remainder of the 20 breast cancers found, were from 12 case reports that resulted in 16 cases of breast cancers (Cormen et al., 2016; Gooren et al., 2015; Sattari et al., 2015; Teoh et al., 2015; Gondusky et al., 2015; Maglione et al.,

2014; Pattison et al., 2013; Dhand & Dhaliwal, 2010; Grabellus et al., 2005; Ganly, 1995; Pritchard et al., 1988; Symmers, 1968). In summary, from the case reports and cohort studies we can deduce a total of 7,453 transsexual persons were included, and 20 were found to have breast cancer.

All cases were of epithelial breast cancer, half of them consisting of invasive carcinomas (50%); adenocarcinomas (15%); ductal carcinomas (10%); unspecified breast carcinomas (10%); ductal carcinomas in situ (5%) and secretory carcinomas. According to Joint and colleagues (2018) in the MtF cohort there were 17 cases with ER positivity (52.9%), 15 cases with PR positivity (40%) and three cases that were receptor negative. BRCA testing was done on five cases, with one testing positive for the BRCA2 mutation. From the 20 cases of breast cancer, 19 patients were on HT before their diagnosis, with the duration ranging from 6 to 37-years. There were eight cases that described the effect of breast cancer diagnosis in their continuation of HT, with five deciding to fully stop HT and two deciding to continue, while one was recommended to stop but further details were not found. Sixteen of the 20 cases mentioned their discussion of treatment path, however one died before beginning their treatment, and another discharged herself before starting treatment, while the rest (14) decided to do treatment.

Stromal Density

One of the most important and strongest risks for breast malignancies in ciswomen is an increase in breast density (Byrne et al., 1987). Density in the breast involves the microenvironment comprised of the stroma and fibroglandular tissue, which aid in the growth and maintenance of the epithelia (Modern surgical pathology CH 19). McCormack and Silva (2006) conducted a meta-analysis on mammogram data from 42

reports, with a total of over 14,000 cases of breast cancer and 226,000 non-cancerous mammogram (controls). The authors set to examine the possible risk associated with breast density and breast cancer in patterns of mammogram studies. Upon finishing their examination, they concluded that strong linear trends appeared with density, thus, defining increase in density of breast tissue as a risk factor for breast cancer. Similar studies arrived at the same conclusions, that increased density is indicative of risk for breast cancer (Dontchos et al, 2015; Ursin et al., 2005; Clemons & Goss, 2001; Li et al., 2005; Brinton et al, 1991; Byrne et al., 1987), but more specifically, Boyd et al. (2002) stated that denser breasts in women are shown to have more than a 4-fold risk of breast cancer.

Research and evidence of breast cancer risks in ciswomen with denser breasts continues to increase and is of the main concerns for clinicians when diagnosing, aside from sex and age. In regards to transwomen, a widely used study by current researchers, Weyers et al. (2010), stated that approximately 60% of the transwomen in their study had "very dense" breasts. Their study included 50 Dutch transwomen, where density in those individuals was due to the presence of more than 25% dense tissue seen in mammography. According to Phillips et al (2014) transwomen develop a wide variety of breast tissue, with more individuals than not demonstrating higher density. Their study on mammography images of MtF individuals, recommended that imagers (radiologists) be cognizant of the impact of long-term use of estrogen on breast density in trasnwomen, because it is not the same breast tissue seen in males with gynecomastia, which is often used as a reference due to higher levels of estrogen seen.

Additionally, breast density has been shown to prevent the ability to detect malignancies in mammogram images (Kerlikowske et al., 1996). In terms of mammogram imaging, density is the proportion of white (in appearance) sections (connective tissue), relative to the dark regions (fatty tissue). Sensitivity of mammogram screening is reduced in the presence of increased density, which causes inaccurate readings of mammograms and possible misdiagnosis of tumors (Corney et al., 2003). For women whose breasts is "fatty" (appeared dark in imaging) mammogram sensitivity is approximately 87% with specificity of 96.6% (Corney et al., 2003). However, in women with very dense breasts, sensitivity is approximately 62.9% and specificity 89.6% (Corney et al., 2003). Thus, denser breasts are shown to increase the risk of breast cancer, and also, decrease mammogram sensitivity, which prevent accurate detection of breast malignancies, which can lead to poorer outcomes.

Other Potentially Contributing Risk Factors

Health disparities in ciswomen. Most disparities that contribute towards risks of breast cancer focus on racial disparities, which are predominantly seen in black women (Bigby et al., 2004), but have neglected to investigate other racial groups and variables. The *American Cancer Society* (2019) has shown that in the comparisons between men and women, women of color are in fact at a higher risk for breast cancer when matched to their male counter parts. For instance, white men are 100 times less likely to develop breast cancer compared to white women, and black men are also less likely to develop breast cancer when compared to the risk seen in black women. When comparing the risks between black women and white women, mortality rates of breast cancer in black women are higher; these are attributed to lack of adequate screening and diagnosis

(Bigby et al., 2005). Lower rates of screening were seen in black women (Clark et al., 1999), which led to efforts in increasing mammography screening, that resulted in similar rates of mammograms in black women compared to non-black (Willet et al., 2004). However, data demonstrated a 25-30% (Schneider & Epstein, 2002) decrease in self-reported (asking the patient to rate themselves during medical questionnaires and/or clinical interviews) screening within the same population, which coincided with studies that non-white women tend not to follow-up after abnormal results in mammograms (Bigby et al., 2004). The combination of all these studies demonstrates that barriers to access to care and lack of preventive and survival education can increase negative outcomes in populations experiencing these health disparities.

A large amount of evidence implies that cancer disparities in black women are due to lack of screening, which results in later stages of cancer at diagnosis (Daly & Olepade, 2015), and reduced access to care (Newman & Kaljee, 2017). A meta-analysis of geographical mortality rates of breast cancer in the U.S showed that in only four counties were the outcomes for black women optimal (Rust et al., 2016). The authors researched 762 U.S counties to examine the consistent mortality rates seen for black women, because since 1989 mortality rates have declined for all groups except black women. They examined data from counties during 1989-2010 where more than half (54%) demonstrated consistent and unchanging disparities. More specifically, approximately, 1 in 4 (24%) had a pattern of worsening disparities in black versus whites. This study coincided with literature on mortality rates and trends that suggest a contribution of social and structural factors for breast cancer risks seen in geographical variations (Grubbs et al., 2013). Changes to make health care more equitable in

Massachusetts, Delaware and Connecticut reflected positive outcomes in their mortality rates and contributed to the elimination of breast cancer disparities (Sighoko et al., 2013; Hunt & Hurlbert, 2013; Van Deer et al., 2013). Thus, lack of screening in disadvantage populations result in worst cancer outcomes that are due, in large part, to health disparities.

Health disparities in MtF. Transgender patients face numerous obstacles during their transition journey, including lower rates of healthcare coverage, reduced financial means and negative social stigma from their health practitioners. Current health insurance plans typically deny coverage for medically necessary procedures, such as prescriptions for sex steroids for HT, needed for the transition of a transgender patient (Padula et al., 2015). Therefore, financial issues are the most common barriers to health-care access for transgender patients (Bucket et al., 2018). Financial barriers to health care have been demonstrated to in a recent study by Fenn et al., (2014) to be the strongest independent predictor of poor quality of life in survivors of cancer, which in this case, would reiterate the weight of the financial burden in MtF populations and the risks of poorer outcomes when cancer does strike.

Barriers to health insurance coverage place a financial hardship on transgender patients (Padula et al., 2015), which often pushes patients to resort to sources of treatment outside of the medical community for HT (Bucket et al., 2018). The use of non-prescribed HT is common and an ongoing concern, with patients arriving to the emergency department from complications due to needle sharing, pulmonary embolism, deep vein thrombosis (DVT) and hyperkalemia (Kaiser et al., 2016). A recent study n San Francisco by Haan et al. (2015) analyzed data from 314 transwomen and found that

approximately 49% of those on HT were using non-prescribed dosages (68.7% reported being on HT) and only 41% were on it consistently, which can pose a health hazard around breast cancer, if dosage is unknown.

Even if a patient is able to afford medical care, transgender patients face common issues in the hands of health care professionals (Belluardo-Crosby et al., 2012). Education and training for clinicians on providing quality care for transgender patients is profoundly absent (Belluardo-Crosby et al., 2012). Transgender patients have reported being seen by medical providers who did not know how to best provide for them; the providers were unaware of transition-related care or the spectrum of transgender patients (Bucket et al., 2018). Transgender patients also consistently report experiencing bias and stigma from clinicians, in the form of the inappropriate medical records, wrong name and/or pronoun usage, invasive questions and the perpetuation of beliefs in only two genders (Padula et al., 2015). The combination of the lack of education and inexperience of the medical community, conflated with the bias and stigmatization contribute to health disparities and barriers that transgender persons face during their transition (Padula et al., 2015).

HT in MtF. Estrogen intake in an MtF patient is the more common HT used (Moore et al., 2003). The intake of exogenous estrogen by an MtF patient reduces androgen secretion, through the suppression of pituitary gland secretion of gonadotropin hormones (Dittrich et al., 2005). However, there is a widely held belie that the desired androgen suppression is typically not achieved by estrogen alone, so anti-androgens are also prescribed.

A review on the guidelines and HT types and dosages by Unger (2016) showed that the typical estrogen intake by an MtF patient was two to three times higher than dosages typically used in HRT for postmenopausal women. Coinciding with prior notions that HT for MtF individuals was modeled loosely after HRT for postmenopausal women (Moore et al., 2003). Unger's review showed that the most common form of estrogen in HT was in the form of a pill (estradiol: 2-4 mg daily) and/or transdermal patch (estradiolvalerate: 5-50 mg every two weeks). Compared to Ratner and Ofri (2001) review of common types and dosages of estrogen in HRT of postmenopausal women: ethinyl estradiol (0.02 mg PO daily) and transdermal path (0.05 mg, 1 patch twice a week), the difference is uncanny. The use of ethinyl estradiol has been limited and cautioned for usage in MtF individuals because of studies demonstrating the increase of risk thromboembolism and even cardiovascular death (Asscheman et al., 2011). As far as anti-androgens, spironolactone appeared to be the most common form of anti-androgen prescribed in Unger's review.

A recent study by Cuhna et al. (2018) reported that estrogen alone sufficed to lower testosterone levels in MtF patients. The authors examined serum levels of 51 transwomen after being placed on two types of estrogen regimen. Forty-one patients were given 0.625 mg of conjugated equine estrogen and the remaining 10 were placed on 1.25 mg. Cyproterone acetate (anti-androgen) was also given to 43 of the patients; 50 mg to 42 and one was given 100 mg. Authors decided on cyproterone acetate instead of spironolactone citing its implications in a synergistic estrogen effect towards physical changes (Prior et al., 1989). The authors concluded that low dosages of estrogen therapy alone reduced testosterone levels from 731.5 ng/dL to 18ng/dL, and the addition of

cyproterone reduced the levels from 750 ng/dL to 21 ng/dL (no statistical difference). Thus, MtF individuals do not need to be exposed to high dosages of estrogen, which can cause a variety of detrimental and unforeseen consequence in order to achieve feminizing hormone levels equal to that in ciswomen. More importantly, patients in their cohort study developed breast in accordance with stage four and five of the Tanner Stages, "Areola elevated above contour breast, forming 'double scoop' appearance," Areolar mound recedes back into single breast contour with areolar hyperpigmentation, papillae development and nipple protrusion," respectively.

According to Heijer et al. (2017) most guidelines leave the dosage of transitioning steroids in the hands of practitioners. Under the influence of blood samples, clinicians are to monitor side effects of HT while achieving the goal of feminization. Heijer et al. states that the fullest effect of HT is typically seen in the first 2-5-years of use, comparing it to the typical pattern seen during female puberty. After that time frame, HT is to be used for maintenance of the achieved feminization. The authors mention that although feminization is the goal, side effects, such as hypogonadism, from HT are to be closely monitored to ensure that the overall well being of the patient is precedence.

Discussion

Epithelial breast cancers in ciswomen (12%) are an established topic of concern and research, but as of now, current literature does not signal the same for transwomen. Furthermore, in ciswomen stromal tumors make up less than 1% of tumors. Case reports on MtF patients with stromal lesions depict a different notion for this population. Within the last two years, three cases were reported showing the presence of rare stromal lesions. All three tumors, Myofibroblastoma (MFB), Pseduoangiomatous

stromal hyperplasia (PASH) and Phyllodes Tumor (PT) have been described as rare entities to occur in both male and females, with stromal proliferation and possible etiologies linked to aberrant hormonal levels, more specifically estrogen. Ciswomen with stromal tumors comprise less than 1% of breast cancers. Here I have identified three cases of MtF individuals with stromal tumors, and current literature depicts the number of epithelial tumors in MtF women as 20 reported cases. Therefore, the ratio in transwomen seems very different to those in ciswomen.

Considering the intake of sex steroids in these three stromal lesion cases the questions arises, is there reason to expect that the MtF rate of stromal malignancies will be significantly greater than that seen in ciswomen? From the small sample size available through current literature, there are only 20 cases of epithelial breast cancer in MtF individuals reported globally, in comparison to 12% lifetime incidence rate of ciswomen. However, when taking these three stromal lesion cases in comparison to those 20 of epithelial origins, the ratio (3:20) produces a 15% value of stromal incidences, which is more than 15x greater than that seen in ciswomen (less than 1%). This calculation is dependent on the limited published studies, which are composed of a very small sample size. However, this can be something that the medical community could try to track better to determine its validity. In the meantime, research on breast cancer in ciswomen could give insight to the possible contributions of estrogen in these developments.

The majority of breast cancers found in ciswomen are epithelial (99%), ER-positive (80%) and of those, 65% are PR-positive, thus research on hormone receptors is key to better grasp the pathological development of breasts. Hormone receptors are

found distributed among the breast tissue and mediate proliferation and differentiation upon activation from sex steroids (estrogen and progesterone). Considering that MtF patients are essentially prompted to undergo female puberty upon the introduction of HT, the absence of higher numbers in epithelial cancers is confounding. Estrogen in ciswomen has been labeled as the possible trigger for neoplastic growth, why aren't MtF patients' demonstrating epithelial breast cancer incidence rates equal to those found in ciswomen? Two obvious possibilities include, one, the health disparities and aversion to care (or lack of adequate care) of the trans community are disguising a larger problem. Secondly, the duration of estrogen exposure for most transwomen today is still short compared to a postmenopausal ciswoman, who doesn't reach peak breast cancer age risk until she has had 35 years of estrogen exposure and then lived another 20 years. Very few transwomen have been using HT for 35 years, and fewer began their transition 55 years ago, therefore, individually or a combination of both could contribute to low rates seen in data today. Why do I think rates will go up with additional data?

I think there are several reasons to expect the incidence of breast cancer in the MTF community to rise. First, HT is more widely accepted as a treatment for MtF and gender dysphoria, therefore, it is reasonable to expect that more transgender persons will begin receiving appropriate HT beginning as early as puberty (Unger, 2016). This increase in lifetime estrogen is expected to increase incidence because women in general have higher incidences than men (100x), and even the short term intake of exogenous estrogen in HRT by postmenopausal has been stated to cause a 2.3% increase risk in breast cancer development (The Hormonal Group, 1997). Secondly, most MtF HT is 2-3x higher in concentration than HRT for postmenopausal women, again, increasing

potential risk. Third, denser breast tissue has been established to increase the risk of breast cancer by a 4-6-fold. Both MtF and HRT users have been noted to have denser breast tissue, 60% and 75% respectively. Fourth, is medical access for MtF improving (for example, are some insurance plans covering treatment?) If so, this will likely increase the number of trans women with access to hormones, increasing the population. Fifth, although

MtF patients are typically compared to ciswomen for control purposes, it is important to examine the possible relationships in males with increased estrogen. A 50-fold increase of breast cancer risk has been noted for individuals Klinefelter Syndrome (KS); the risks are attributed to the increase levels of estrogen in the estrogen to testosterone ratio. KS individuals have higher levels of estrogen and lower testosterone due to the extra X-chromosome and have excess peripheral conversion of available testosterone into estrogen. MTF women have a similar profile due to taking estrogen and androgen blockers. Sixth, another estrogen-driven disorder in males is gynecomastia, where coincidentally, PASH tumors are closely associated to develop on lie of gynecomastia in males (PASH tumor is one of the stromal lesions found in the case reports of MtF stromal malignancies). Gynecomastia is due to an increase of estrogen in males. Although these patients have not been identified to be at risk for breast cancer, males with gynecomastia are at risk to develop testicular cancer, which develops due to increase estrogen levels, indicating that estrogen-sensitive tumors can arise in XY individuals exposed to estrogens.

In regards to epithelial breast cancer rates in MtF, my assessment is compromised due to the small sample size that is available in the literature. Since the sample size of

transgender persons is small, it could be that the rate for epithelial breast cancer is too low or too high. However, right now, with all the other information I found, I believe that number is too low, because the rate of breast cancer in MtF is only 4-fold greater than cismen, which are not under the influence of exogenous estrogen. I think that the population of transwomen will expand, because I believe there are more people that identify as transgender today, but, either do not have access to care or do not feel safe and secure to seek care. When financial barriers are removed and more MtF individuals can afford HT; and as our society, as a whole, becomes more accepting and inclusive towards transgender individuals; and as more medical providers are better trained to provide adequate care, the transgender population will expand. As the population of transgender persons grows, trasnwomen will be on HT longer and we will have more data to accurately depict the correct rates of breast cancer in this population. These observations reflect a correlation between estrogen and breast cancer risks, but all of the trends point in the same direction. Hopefully this observation will encourage possible research approaches in the future.

In the stromal cases found in MtF patients again we find a small sample size that could affect the actual rates. There are reason to expect this rate is higher, for instance, there is a high percentage of stromal density found in transwomen and when compared to the statistics of breast cancer risks in ciswomen, denser breasts are considered to have a 4-6-fold increase risks of breast cancer (McTiernan et al., 2005). Another reason can be due to the small amount of overall information we have on stromal malignancies in the general population that can contribute to a decrease in awareness in medical providers. If little is known about stromal lesions and medical providers rarely see these lesions in

cismen or ciswomen, when compounded with the lack of education in medical providers for the care of MtF persons, this attributes to a decrease in findings and reports of stromal lesions in MtF persons. Amounts and types of estrogen for HT in MtF patients are not under guidelines or strict monitoring by medical institutions, which could also contribute to higher rates in the future. The rate of stromal lesions in MtF could also be lower, because stromal lesions are a rare entity in ciswomen, the population with most exposure to estrogen in their lifetime, but that is the point of a small sample size. The small sample size of transwomen with stromal lesions could be a sampling error, that could be an error on the high end of the spectrum or the low end, but the only way to decide is to have bigger numbers. Along with the financial obstacles, lack of education in clinicians and stigmatization from providers, the health disparities that this population face, further conflate this topic.

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